EXHIBIT A

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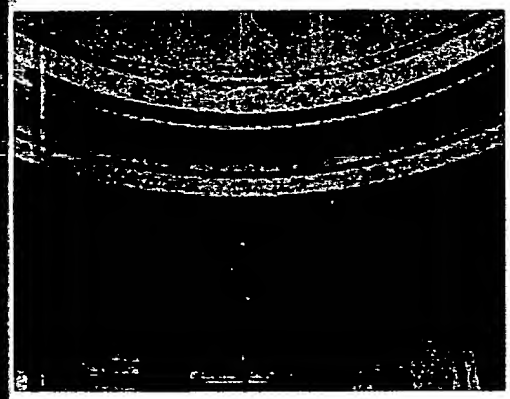

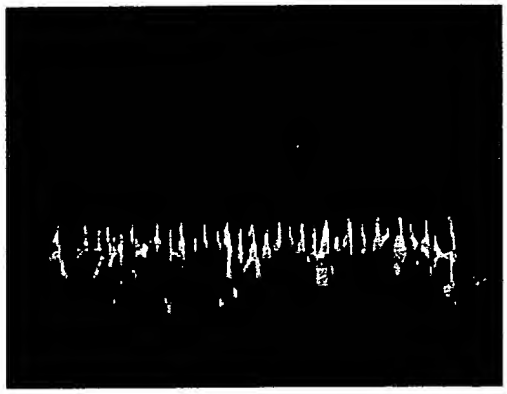

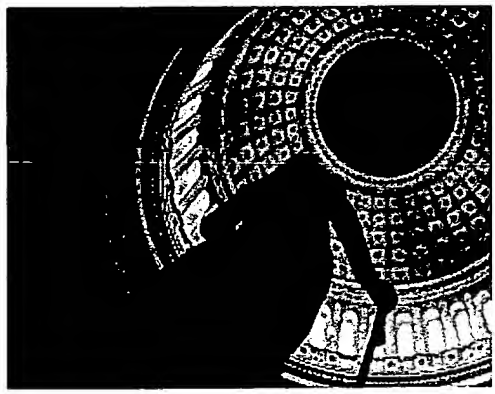

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The ratio between basal and induced condition was two orders of magnitude and a very low transgene expression was detected in the "off" condition. We then tested the performance of this vector *in vivo*. We injected the new self-regulating LV expressing hFIX into the tail vein of SCID mice. Mice were treated or not with Dox in the drinking water and bled periodically to measure hFIX expression in the plasma by ELISA. The animals that were maintained in the "on" condition stably expressed hFIX up to levels within the therapeutic range for hemophilia treatment, while hFIX expression was undetectable in the animals kept in the "off" condition. Cycles of Dox treatments performed on the same animals induced the expected changes in hFIX expression with subsequent cycles showing the same basal and induced level of expression, indicating stable maintenance of the vector and robust performance over time*in vivo*. In conclusion, the optimised self-regulating LV represents a powerful tool to address major safety and efficacy requirements of gene therapy.

1191. Peptide-Mediated Delivery of an Artificial Transcription Factor to Upregulate Specific Endogenous Gene Expression: A Novel Approach to Gene Therapy

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Cys2-His2 zinc finger proteins (ZFPs) are among the most highly represented structural motifs in the human genome. The relationship between ZFP structure and DNA binding specificity has been elucidated well enough to allow *de novo* design of artificial ZFP transcription factors capable of regulating virtually any endogenous gene. Here we show that *peptide-mediated* delivery of an artificial ZFP engineered to activate the VEGF-A gene (VEGF-A ZFP) can be used to upregulate endogenous gene expression both *in vitro* and *in vivo*. Peptide phage display (PPD) was used to identify multiple unique short peptide sequences capable of crossing endothelial barriers and internalizing into cells. Using site-directed insertional mutagenesis, we subcloned multiple of these identified peptide internalization sequences (IS) onto the 5' end of VEGF-A ZFP. Recombinant fusion proteins in which the peptide targeting sequences were linked to VEGF-A ZFP were then expressed and purified. Quantitative RT-PCR of NIH 3T3 mouse fibroblasts transduced with the fusion protein shows statistically significant upregulation of VEGF-A mRNA levels (259%, 260%, 276% at 1, 2, and 3 hours respectively) when compared to delivery of the IS alone ($p<.005$). Furthermore, our PPD-derived amino acid sequences are more efficient at internalization than the previously defined protein transduction domain antennapedia. Messenger RNA levels of the three major VEGF-A endogenous splice variants 120, 164, and 188 are also upregulated (3.4- 3.6- and 1.6-fold respectively) which mimics the native biologic complexity of the cell and may play a critical role in creating a mature vascular phenotype. We have visually localized fluorescently labeled IS-VEGF-A ZFP in both the cytoplasm and the nucleus thereby confirming successful cellular internalization and nuclear targeting. Finally, one hour after a single injection of IS-VEGF-A ZFP into mouse hindlimb skeletal muscle, we are able to show a 2.9-fold increase in VEGF-A mRNA levels when compared to delivery of IS alone and, for the first time, demonstrate that *peptide-mediated* delivery of an artificial transcription factor can upregulate specific VEGF-A endogenous gene expression *in vivo*.

1192. R bust Activati n of Vascular Endoth lial Gr wth Fact r A Using Design d Zinc Finger Protein Transcripti n Factors

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Gene therapies that use designed transcription factors to regulate a patient's own endogenous genetic loci may offer significant benefits over cDNA-based approaches. Demonstrated advantages include the capacity to upregulate all splice variants of a therapeutic gene in their natural proportions (1, 2) and to impose ligand-regulatable protein expression using a single introduced transgene (3). We are developing designed Zinc Finger Protein (ZFP) transcription factors capable of regulating the endogenous Vascular Endothelial Growth Factor A (VEGF-A) locus as pro-angiogenic agents for treatment of cardiovascular disease. In previous studies, we had designed a panel of ZFP transcription factors targeted to the VEGF-A locus and had demonstrated the capacity of these proteins to upregulate VEGF-A expression in HEK-293 cells (1) and to induce angiogenesis in the mouse ear (2). These studies demonstrated the potential for designed ZFP transcription factors to serve as pro-angiogenic therapeutics, as well as tools for studying VEGF-A function. We have now extended our studies to further explore two features of these VEGF-A-activating ZFPs that may impact on their ultimate utility: the range of cell types in which these factors can function and the magnitude of VEGF-A upregulation. First, to directly test for cell-type dependent function, several designed ZFP-transcription factors were tested for ability to activate the VEGF-A locus in a wide range of cell types. These studies revealed VEGF-A activation in all systems tested. Second, to gauge the variability of VEGF-A locus architecture and its accessibility to transcription factors, DNase 1 accessibility mapping was conducted in over 20 cell types. These studies revealed a consistent pattern of enhanced DNase 1 accessibility, suggesting a constitutively accessible and conserved chromatin architecture at the VEGF-A locus. Finally, to better gauge VEGF-A activation magnitude, we created stable cell lines bearing a doxycycline-inducible ZFP transgene and measured VEGF-A levels produced by these lines upon ZFP induction. The cell lines used for these studies – HEK 293, HeLa, and U-2 OS – each express low to moderate levels of VEGF-A in the absence of ZFP (0.5 to 5 fg VEGF-A [cell·day]$^{-1}$). Upon addition of doxycycline, we found that each line expressed high amounts of VEGF-A (~50 fg [cell-day]$^{-1}$) comparable to levels produced by cell lines that induce robust angiogenesis *in vivo*. These studies demonstrate the potency of our designed ZFP transcription factors and suggest that these proteins will be broadly capable of inducing robust angiogenesis *in vivo*.

1193. Targeting Angiostatic Gene Expression for the Treatment of Ocular Neovascular Disord rs Using a Hypoxia Responsive, EIAV-Based Lentiviral Vector

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Neovascularisation of the retina and choroid is a central feature of diabetic retinopathy (DR) and age-related macular degeneration (AMD) respectively. These conditions are leading causes of blindness in developed countries. Current treatments are of limited efficacy due to their transient and inherently destructive nature.

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